

A glutathione-Sepharose "Pull Down"

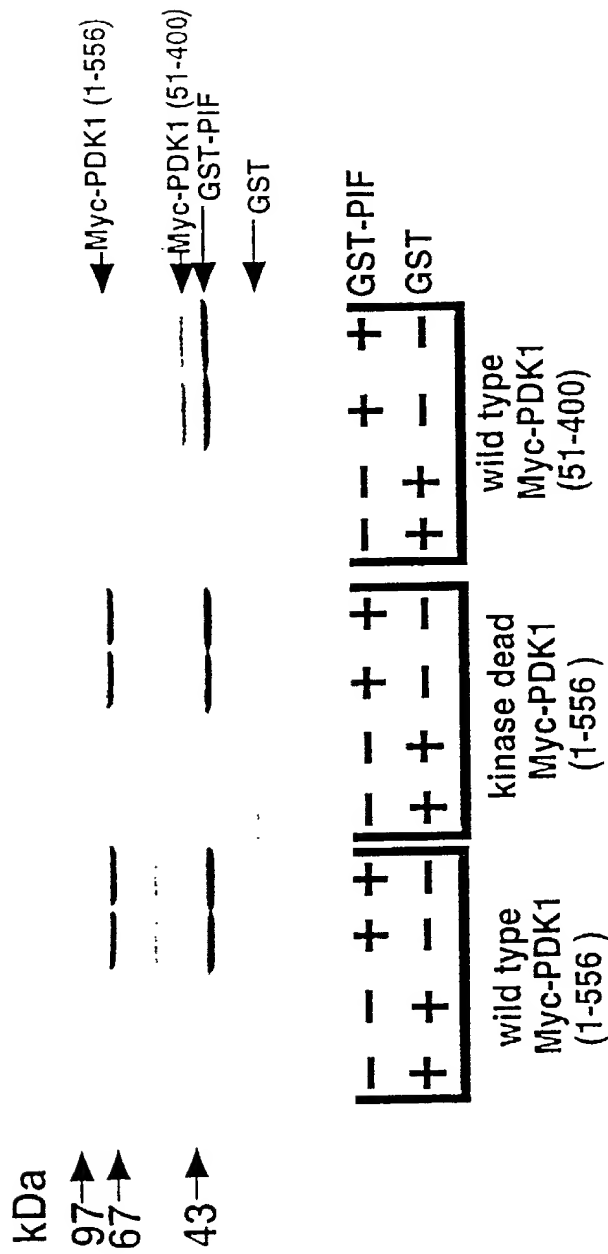


Fig 2 (page 1 of 3)

Myc antibody immunoprecipitate

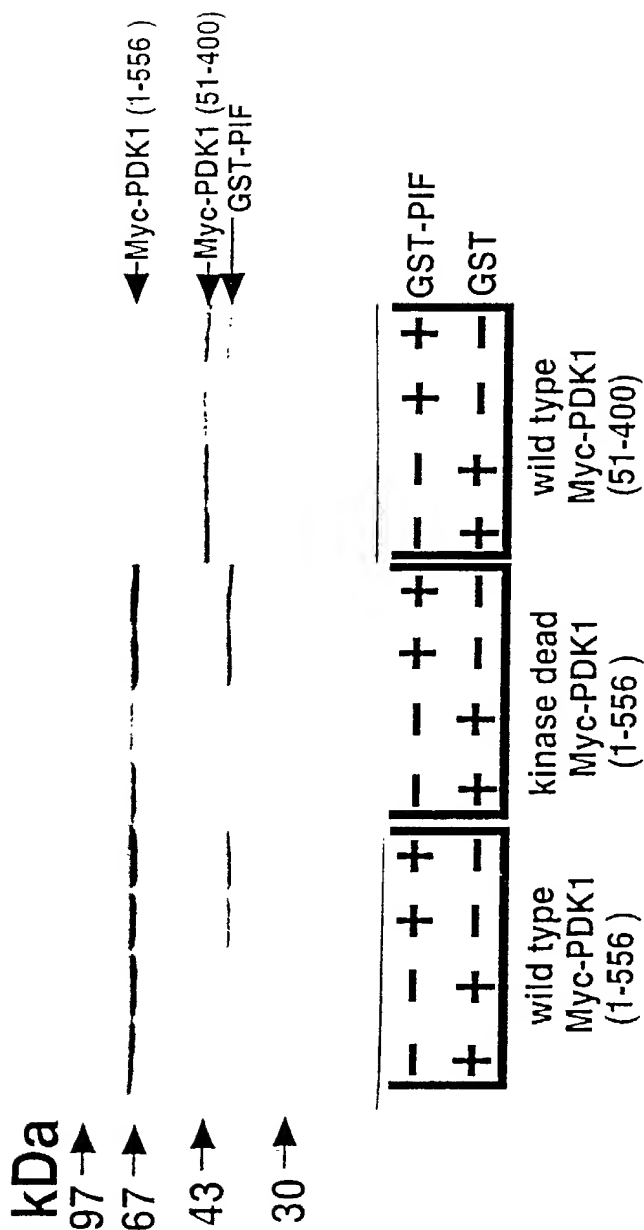
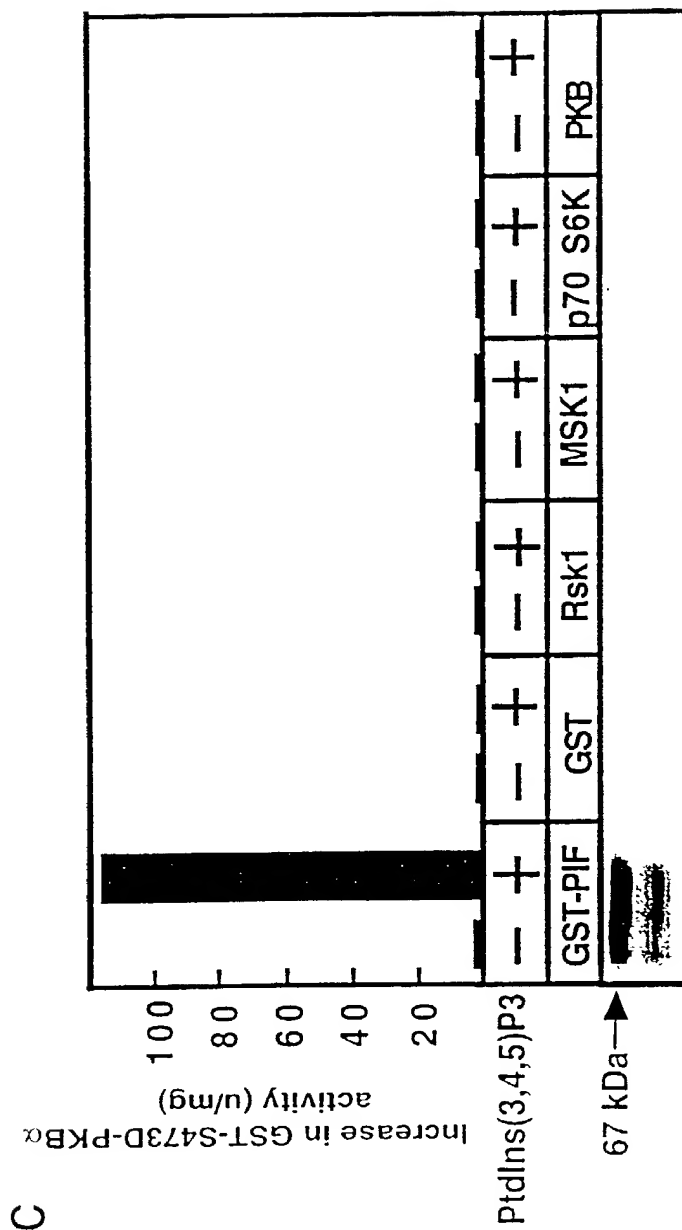


Fig 2 (page 2 of 3)



Western blot for endogenous PDK1

Fig 2 (page 3 of 3)

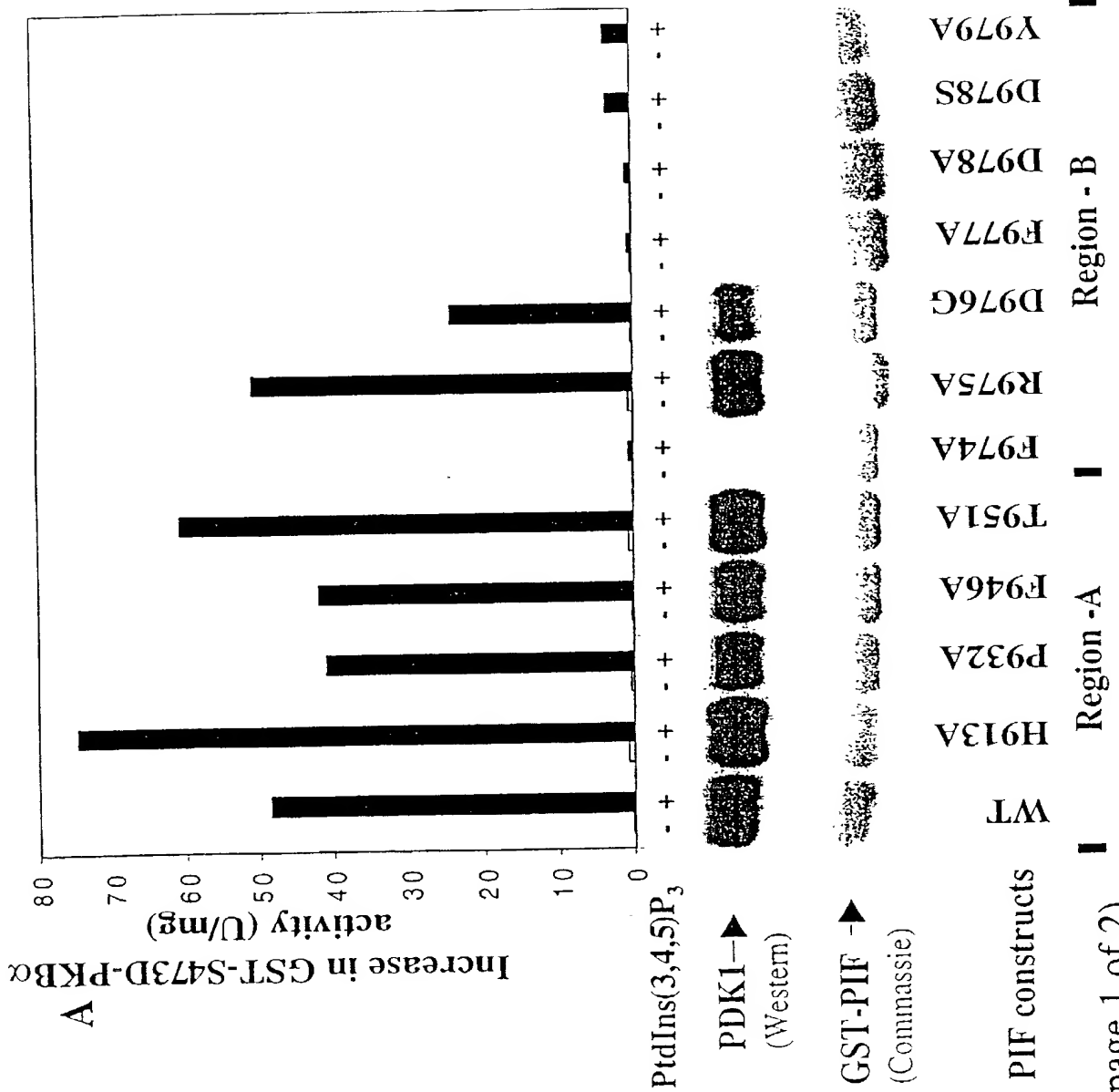


Fig 3 (page 1 of 2)

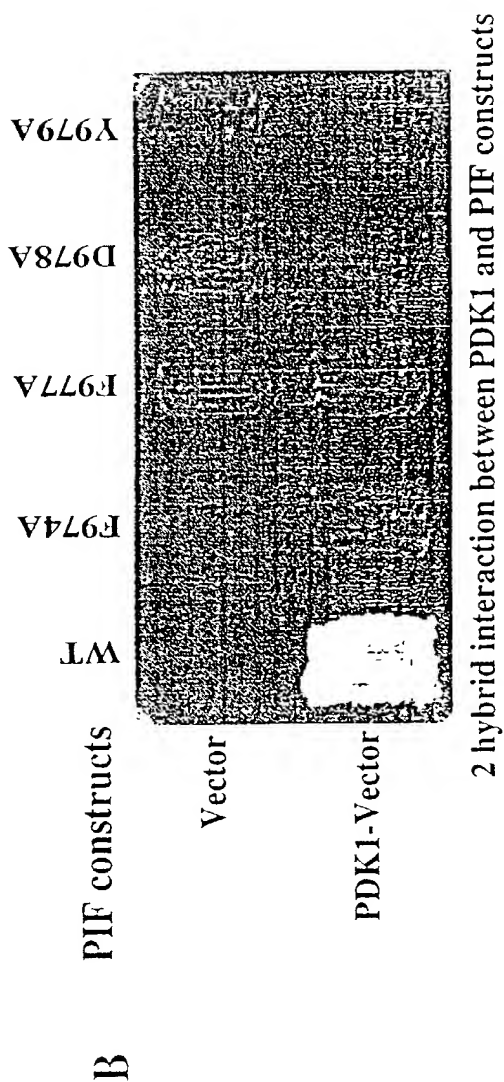


Fig 3 (page 2 of 2)

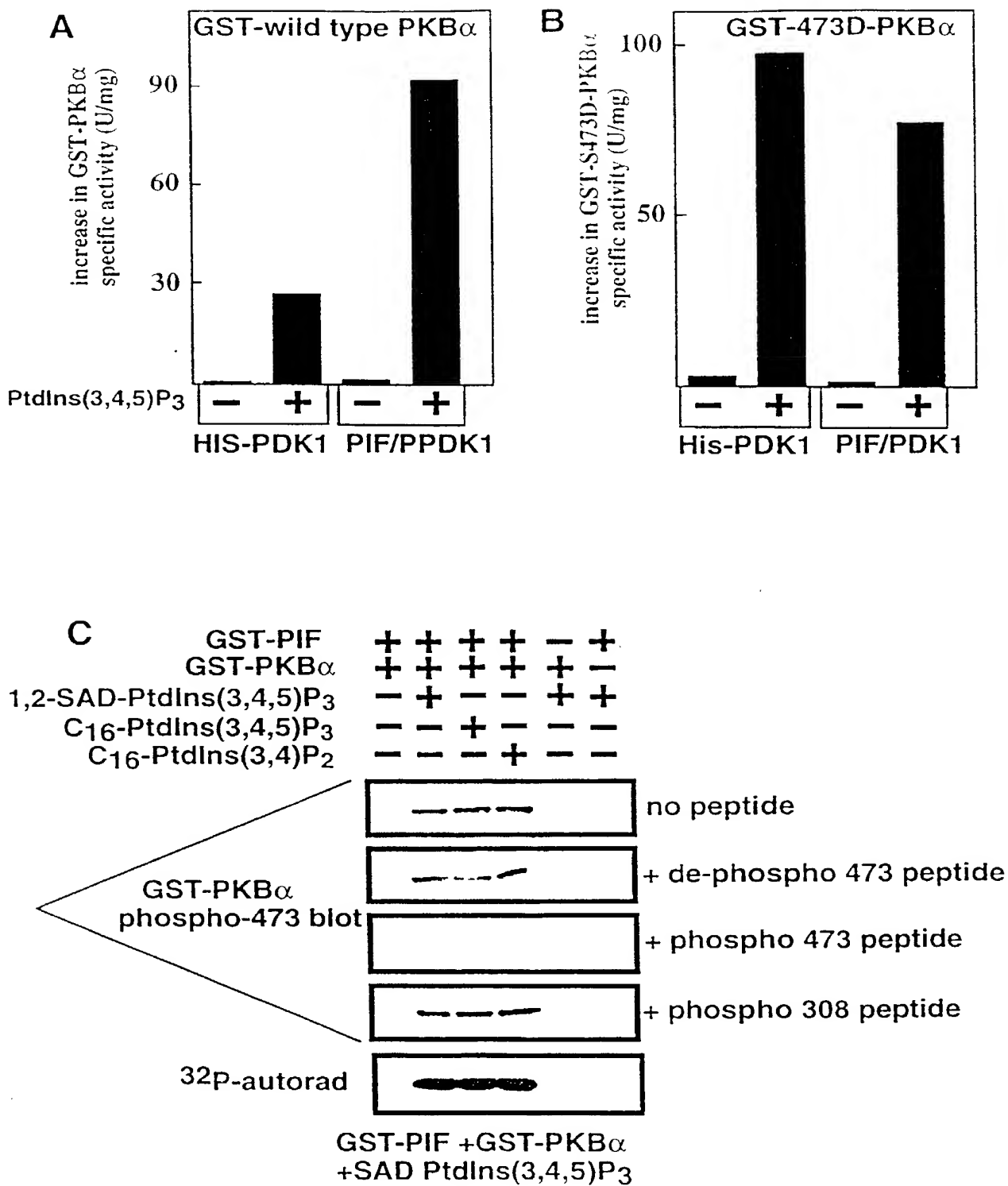


Fig 4 (page 1 of 2)

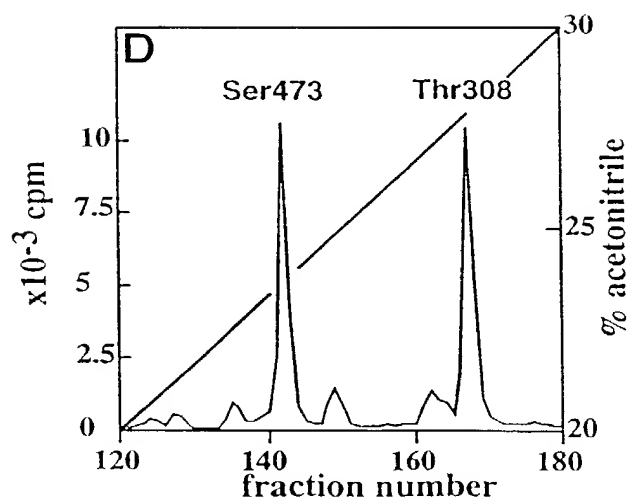


Fig 4 (page 2 of 2)

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A

	Control	Non specific antibody	PDK1 protein antibody	PDK1 peptide antibody	PDK1 peptide antibody + Peptide
1,2-SAD-PtdIns(3,4,5)P ₃	- +	- +	- +	- +	- +
C16-PtdIns(3,4,5)P ₃	- +	- +	- +	- +	- +
C16-PtdIns(3,4)P ₂	- +	- +	- +	- +	- +

PKB α phospho-Ser473 blot in supernatant of immunoprecipitates

B

H913A	P932A	F946A	T951A	Q971A	F974A	R975A	D976G	F977A	D978A	D978S	Y979A
- +	- +	- +	- +	- +	- +	- +	- +	- +	- +	- +	- +

1,2-SAD-PtdIns(3,4,5)P₃

PKB α phospho 473 blot

Region A Region B

935-FRDFDY-940

PKK2 motif in PRK2

Fig 5

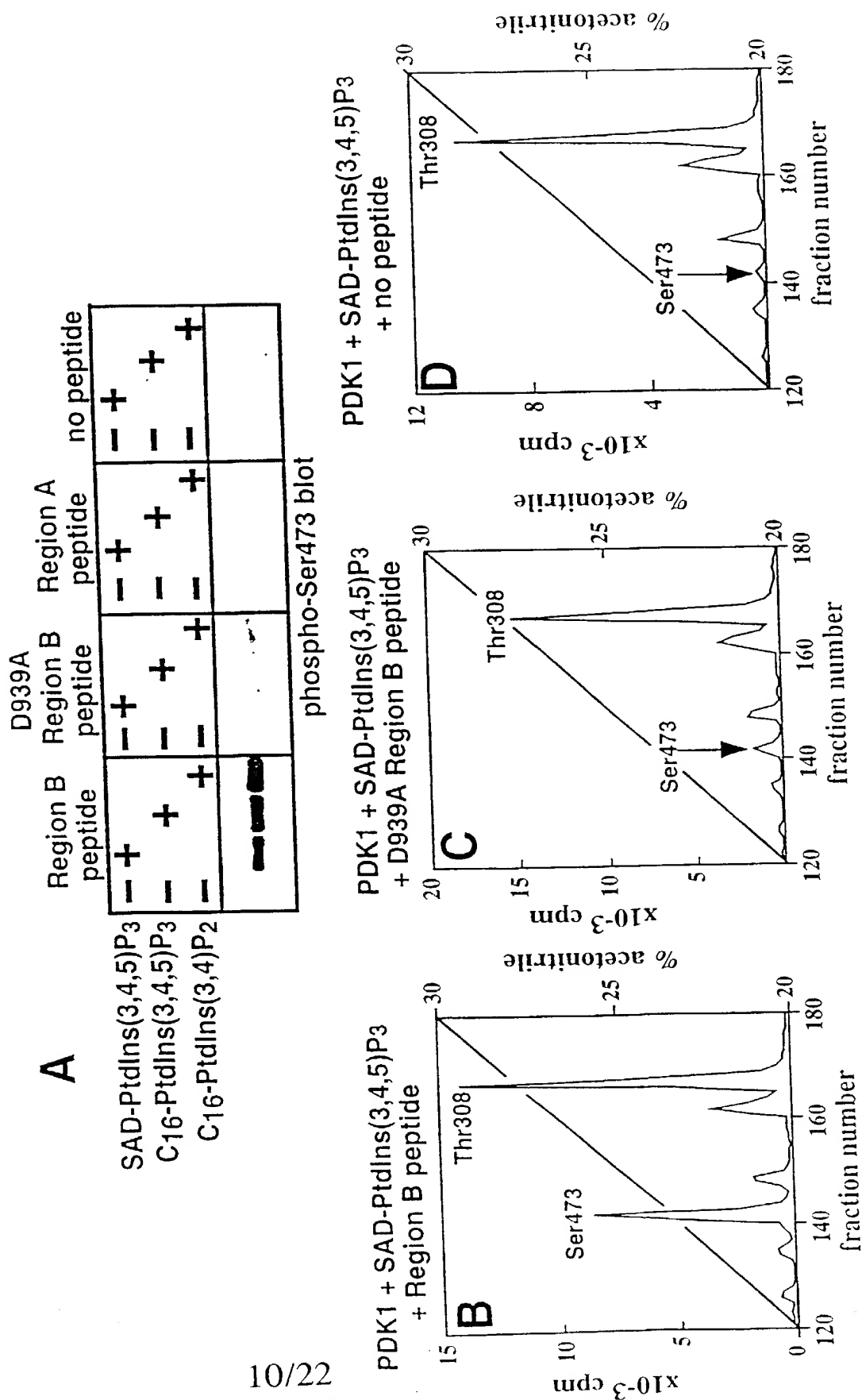


Fig 6

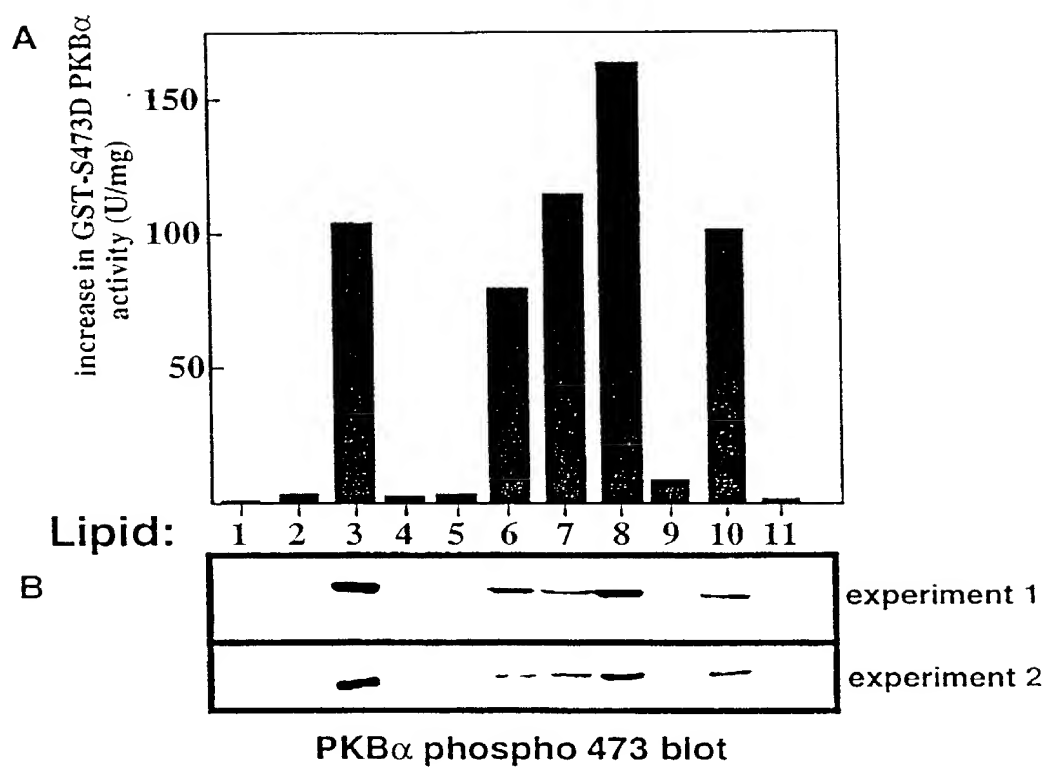


Fig 7

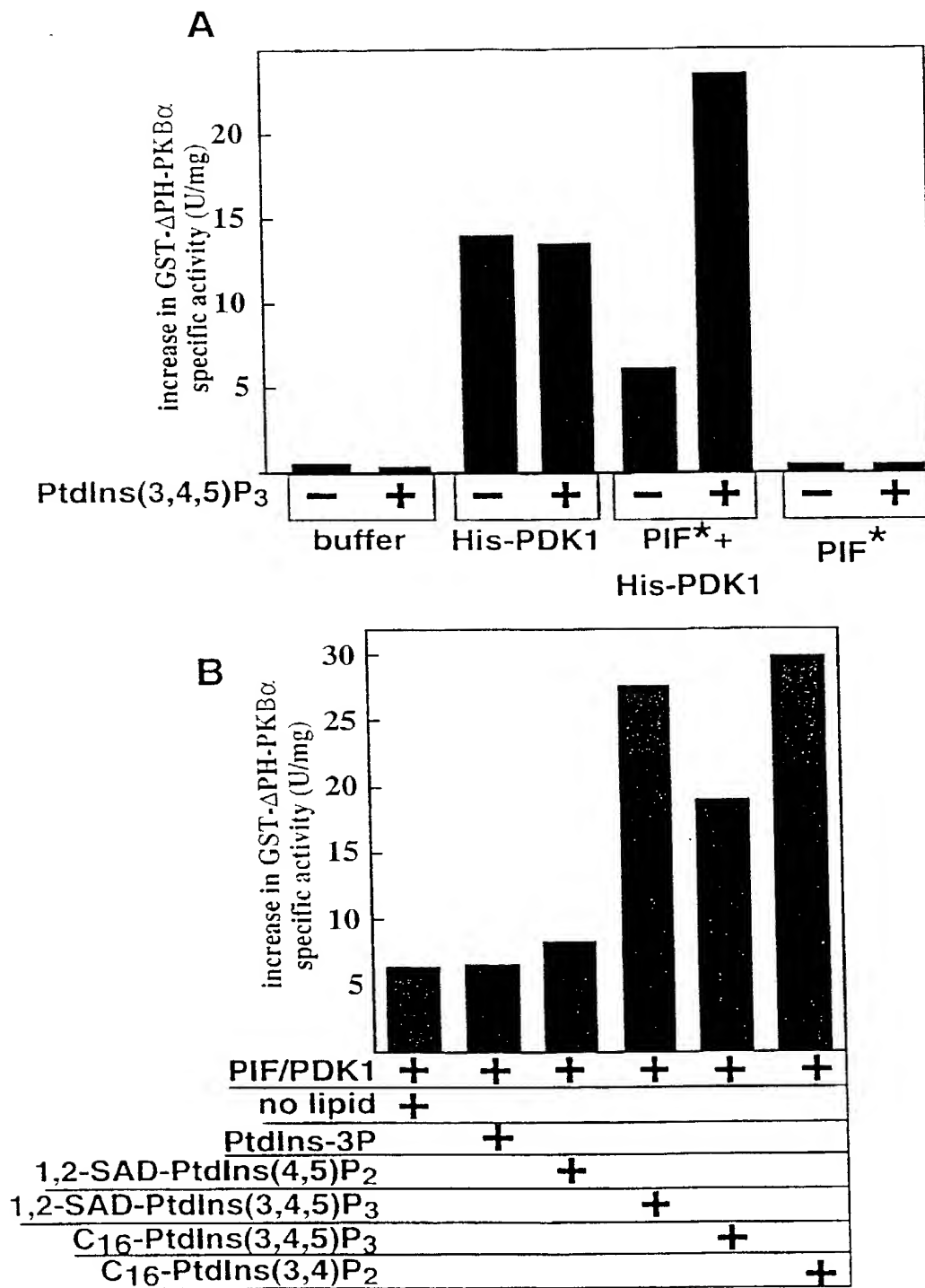


Fig 8

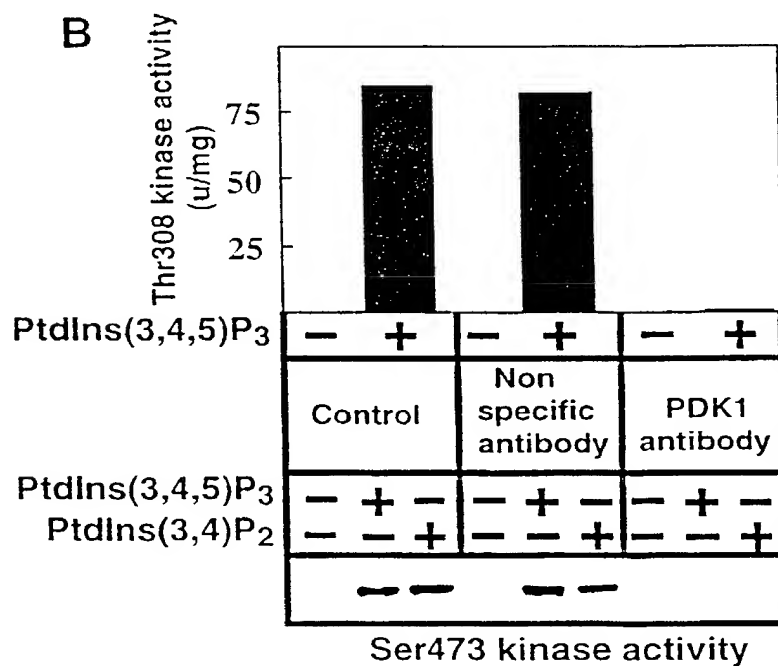
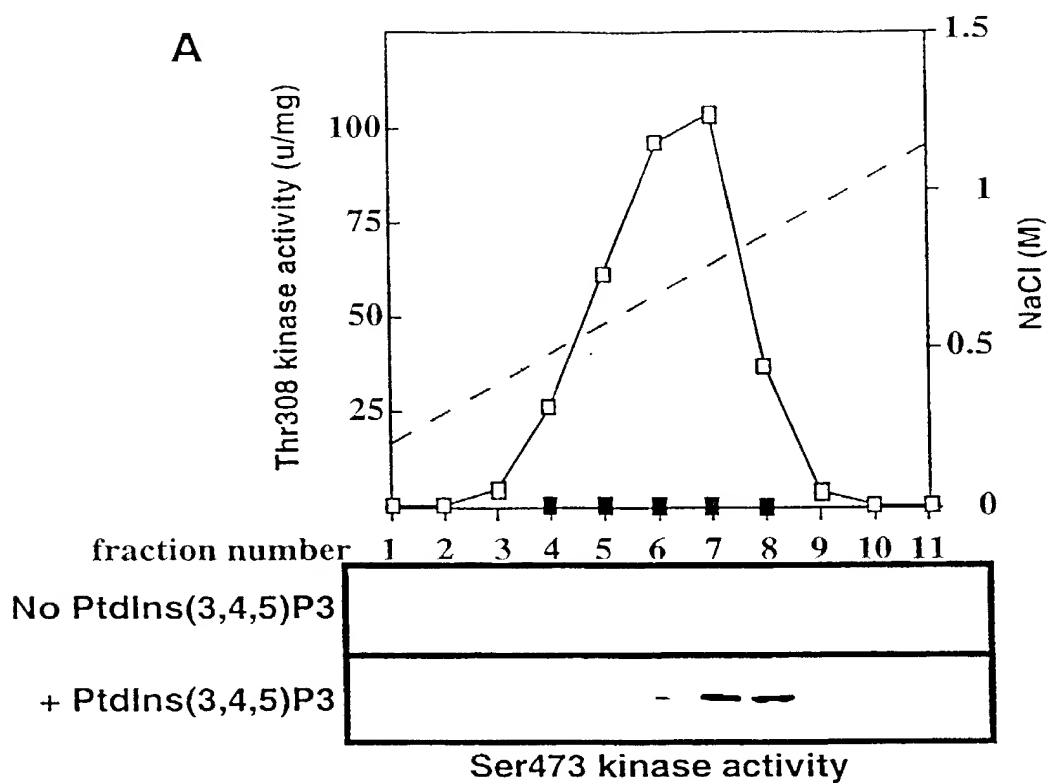


Fig 9

Fig 10

MARTTSQLYDAVPIQSSVVLCSPPSPSMVRTQTESSTPPGIPGGSRQGPAM
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ATSREYAIKILEKRHIKENKVPYVTRERDVMSRLDHPFFVKLYFTFQDDE
KLYFGLSYAKNGELLKYIRKIGSFDETCTRFYTAEIVSALEYLHGKGIHR
DLKPENILLNEDMHIQITDFGTAKVLSPESKQARANSFVGTAQYVSPPELLT
EKSACKSSDLWALGCIIYQLVAGLPPFRAGNEYLIFQKIIKLEYDFPEKFF
PKARDLVEKLLVLDATKRLGCEEMEGYGPLKAHPFFESVTWENLHQQTTPPK
LTAYLPAMSEDDDCYGNYDNLLSQFGCMQVSSSSSSSHLSASDTGLPQRS
GSNIEQYIHDLDNSNFELDLQFSEDEKRLLEKQAGGNPWHQFVENNLILK
MGPVDKRKGLFARRRQLLLTEGPHLYYVDPVNKVLKGEIPWSQELRPEAKN
FKTFFVHTPNRTYYLMDPSGNAHKWCRKIQEVWRQRYQSHPDAAVQ

PDK1 sequence

Fig 11 (page 1 of 2)

Human PRK2 sequence information:

LOCUS 1000125 984 aa 04-FEB-1999
 DEFINITION PRK2
 ACCESSION 1000125
 PID g1000125
 DBSOURCE GENBANK: locus HSU33052, accession U33052
 KEYWORDS
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (residues 1 to 984)
 AUTHORS Palmer, R.H., Ridden, J. and Parker, P.J.
 TITLE Identification of multiple, novel, protein kinase C-related
 gene products
 JOURNAL FEBS Lett. 356 (1), 5-8 (1994)
 MEDLINE 95080426
 REFERENCE 2 (residues 1 to 984)
 AUTHORS Palmer, R.H., Ridden, J. and Parker, P.J.
 TITLE Cloning and expression patterns of two members of a novel
 protein-kinase-C-related kinase family
 JOURNAL Eur. J. Biochem. 227 (1-2), 344-351 (1995)
 MEDLINE 95154310
 REFERENCE 3 (residues 1 to 984)
 AUTHORS Palmer, R.H.
 TITLE Direct Submission
 JOURNAL Submitted (02-AUG-1995) Ruth H. Palmer, Protein
 Phosphorylation,
 ICRF, 44 Lincoln's Inn Fields, London, WC2A 3PX, UK
 COMMENT Method: conceptual translation.
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 /db_xref="taxon:9606"
 /clone_lib="cDNA library from human DX3 cell line
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 Protein 1..984
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 CDS 1..984
 /note="lipid-activated, protein kinase C-related,
 serine/threonine protein kinase"
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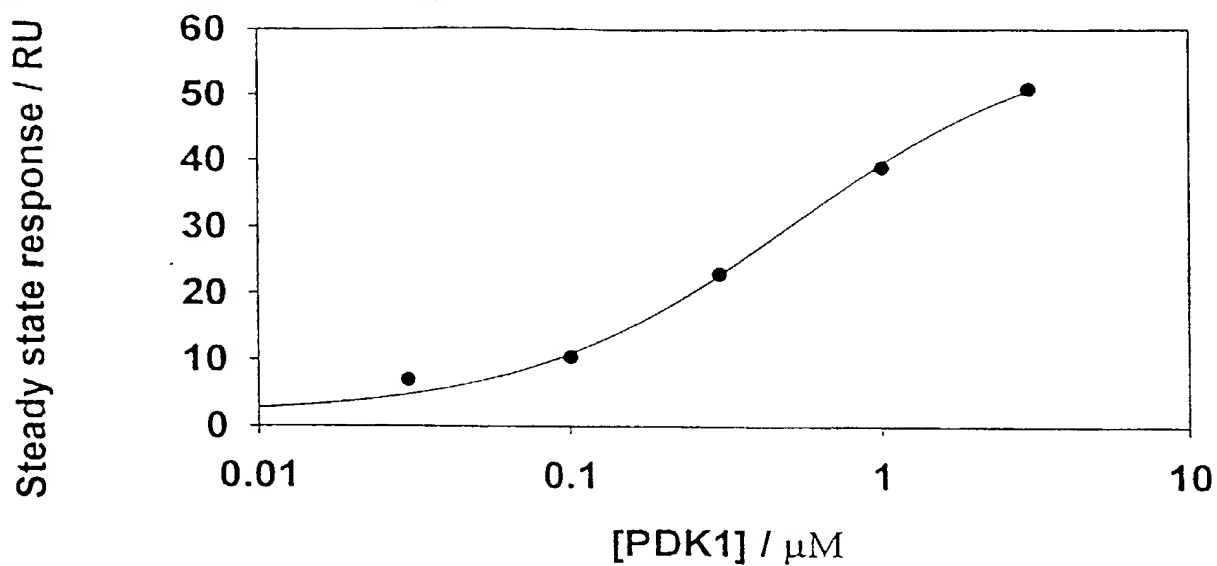
ORIGIN

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 121 tpdtpnnndpr cstsnrika lqkqldielk vkqgaenmiq mysngsskdr klhgtaqql
 181 qdsktkievi rmqilqavqt nelfadnakp visplelrme elrhhfrief avaeagaknm
 241 klhgsgkvtd rkalseaqar tnessqkldi lkysleqrln evpknnoksr lileelslva
 301 asptlsprqs mistqnqyst lskpaaltgt levrlmgcqd ilenvpqrsk atsvallpgws
 361 psetrssfms rtsksksgss rnllktddls ndvcavlkld ntvvgqtswk pishqswdqk
 421 ftleldrsre leisvywrw rslcavkflr ledflonqrh gmclylepqg tifaevtffn

481 pvierrpklq rqkkifskqq gktflrāpqm niniatwgrl vrraiptvnh sgtfspqapv
541 pttvpvvdvr ipqlappasd stvtkldfdl epepppappr asslgeides selrvldipg
601 qdsetvfdiq ndrnsilpks qseykpdtpq sgleysgiqe ledrrsqqrq qfnlqdfrc
661 avlgrgntgk villaeykntn emfaikaikk gdivardevd slmcekrife tvnsvrhpf
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781 rdlkldnlll dtegfvkiaf fglckegmgy gdrststfcgt peflapevlt etsytravdw
841 wglgvliiem lvgespfpgd deeevfdsiv ndevryprfl steaisimrr llrrnperri
901 gasekdaedv kkhpfrrlid wsalmdkkvk ppfiptirgr edvsnfdded tseapiltpp
961 repriiseee qemirddfyi adwc

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(A) HisPDK1 binding to GST-PIF



(B) HisPDK1 binding to PIF region B peptide

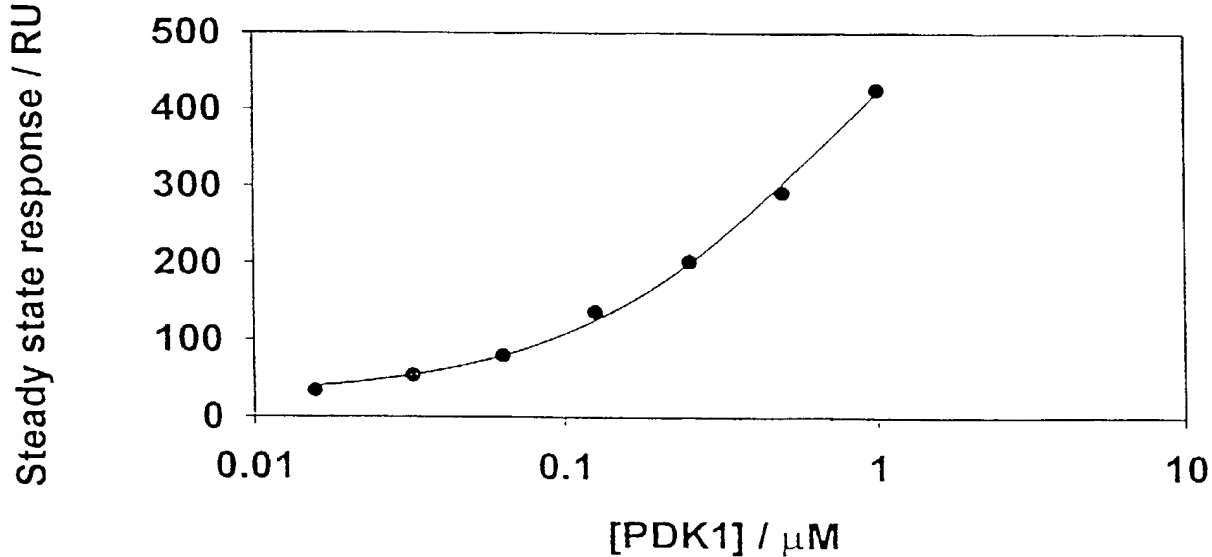


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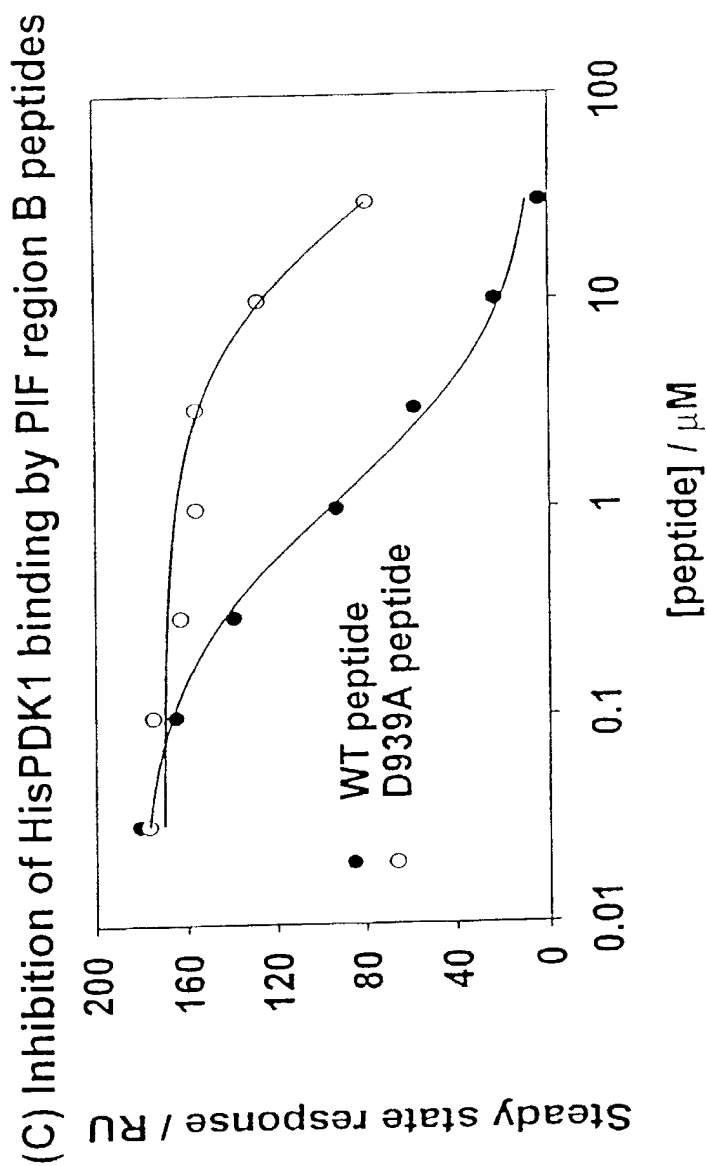



Fig 12 (page 2 of 2)

A

	Wild type GST-PDK1			kinase dead GST-PDK1		
GST-PIF*	+	+	+	+	+	+
SAD-PtdIns(3,4,5)P ₃	+	+	+	+	+	+
C16-PtdIns(3,4,5)P ₃	-	+	+	-	+	+
C16-PtdIns(3,4)P ₂	-	-	+	-	+	+
						

phospho-Ser473 blot

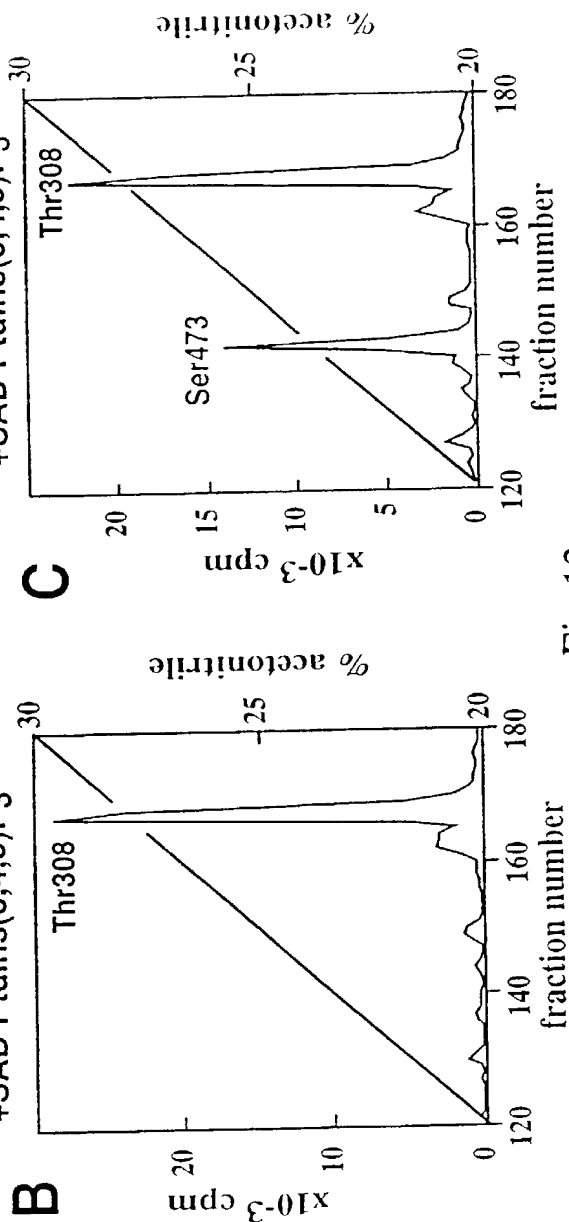


Fig 13

Fig 14

Human PRK1 sequence information:

LOCUS 2136028 942 aa 02-JUL-1996
 DEFINITION protein kinase PRK1 - human.
 ACCESSION 2136028
 PID g2136028
 DBSOURCE PIR: locus I53327
 summary. #length 942 #molecular-weight 103989 #checksum 8328.
 PIR dates: 02-Jul-1996 #sequence_revision 02-Jul-1996
 #text_change 02-Jul-1996.
 KEYWORDS .
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Mammalia, Eutheria; Primates; Catarrhini;
 Hominidae; Homo.
 REFERENCE 1 (residues 1 to 942)
 AUTHORS Palmer, R.H., Ridden, J. and Parker, P.J.
 TITLE Cloning and expression patterns of two members of a novel
 protein-kinase-C-related kinase family
 JOURNAL Eur. J Biochem. 227 (1-2), 344-351 (1995)
 MEDLINE 95154310
 FEATURES Location/Qualifiers
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 /organism="Homo sapiens"
 /db_xref="taxon:9606"
 Protein 1..942
 /product="protein kinase PRK1"

ORIGIN

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 121 satnlrsrvag lekqlalelk vkqgaenmiq tysngstkdr kliltaqqml qdsktkidli
 181 rmqlrralqa dqlenqaapd dtqgspdlga velrieelrh hfrvehavae gaknvlrlis
 241 aakapdrkav seaqekltres nqklgllrea lerrlgelpa dhpkgrrllre elaaassaaf
 301 strlagpfpa thystlckpa pltgtlevrv vgcrdlpeti pwnptpsmgg pgtpdsrppf
 361 lsrparglys rsgslsgrss lkaeaentse vstvlkidnt vvgqtswkpc gpnawdqsft
 421 lelerarele lavfwrdrqg lcalkfikle dfldnerhev qldmepggcl vaevtfrnpv
 481 ieriprlrrq kkifskqgk afqrarqmni dvatwvrlir rlipnatgtg tfspgaspgs
 541 earttgdisv eknlgtddsd sspqkssrdp psspslssp igestapelp setqetpgpa
 601 lcsplrkspl tledfkflav lgrghfgkvl lsefrpsgel faikalkkgd ivardevesl
 661 mcekrilaav tsaghpflvn lfgcfqtpeh vcfvmeysag gdlmlhihsd vfsepraify
 721 sacvvlgqlqf lhekivyr d lkldnllldt egyptkiadfg lckegmgygd rtstfcgtpe
 781 flapevltdt sytravdwg lgvillyemlv gespfpgdde eevfdisvnd evryprflsa
 841 eaigimrrll rnpperrlgs serdaedvkk qpffrtlgwe allarrlppp fvptlsgrtd
 901 vsnfdeeftg eaptlspprd arpltaaeqa afldfdvav gc

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Human PKC zeta sequence information:

LOCUS 478322 592 aa 28-FEB-1997
 DEFINITION protein kinase C (EC 2.7.1.-) zeta - human.
 ACCESSION 478322
 PID g478322
 DBSOURCE PIR: locus JN0877
 summary: #length 592 #molecular-weight 67731 #checksum 87.
 genetic: #gene GDB:PRKCZ ##cross-references GDB:128040.
 superfamily: protein kinase C zeta; protein kinase C zinc-binding
 repeat homology; protein kinase homology.
 PIR dates: 03-May-1994 #sequence_revision 03-May-1994
 #text_change 28-Feb-1997.
 KEYWORDS ATP; phorbol ester receptor; phosphotransferase;
 serine/threonine-specific protein kinase; zinc.
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Mammalia; Eutheria; Primates; Catarrhini,
 Hominidae; Homo.
 REFERENCE 1 (residues 1 to 592)
 AUTHORS Kochs,G., Hummel,R., Meyer,D., Hug,H., Marme,D. and
 Sarre,T F.
 TITLE Activation and substrate specificity of the human protein
 kinase C alpha and zeta isoenzymes
 JOURNAL Eur. J. Biochem. 216 (2), 597-606 (1993)
 MEDLINE 93387312
 REFERENCE 2 (residues 1 to 592)
 AUTHORS Barbee,J.L., Deutscher,S.L., Loomis,C.R. and Burns,D.J.
 TITLE The cDNA sequence encoding human protein kinase C-zeta
 JOURNAL Gene 132 (2), 305-306 (1993)
 MEDLINE 94040779
 REFERENCE 3 (residues 1 to 592)
 AUTHORS Hug, H.
 TITLE Direct Submission
 JOURNAL Submitted (??-SEP-1992) to the EMBL Data Library
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 /organism="Homo sapiens"
 /db_xref="taxon:9606"
 Protein 1..592
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 Region 131..180
 /note="protein kinase C zinc-binding repeat homology
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 /region_name="domain"
 Region 250..518
 /note="protein kinase homology #label KIN"
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 Region 258..266
 /note="protein kinase ATP-binding motif"
 /region_name="region"
 Site 281
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/site_type="active"

ORIGIN

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121 rwrklyrang hlfqakrfnr raycgqcser iwglarqgyr cinckllvhk rchglvpltc
181 rkhdmsvmps qeppvddknc dadlpseetd giayissrk hdsikddsed lkpvidgmdg
241 ikisqglglq dfdlirvigr gtyakvllvr lkkndqiyam kvvkkelvhd dedidwvqte
301 khvfcqassn pflvglhscf qttsrlflvi eyvnggd1mf hmqrqrklpe eharfyaaei
361 cialnflher giiyrdlkld nvlldadgh1 kldygmcke glpggdtst fcgtpnyiap
421 eilrgeeygf svdwwalgv1 mfemmagrsp fdiitdn1pdm ntedylfqvi lekpiriprf
481 lsvkashvlk gflnkdpker lgrcpqtgfs dikshaffrs idwdllekkq alppfqpqit
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Fig 15 (page 2 of 2)